Monoclonal Anti-human EphA2-Phycoerythrin

Catalog Number: FAB3035P
Lot Number: ACSQ01
100 Tests

Reagents Provided

Phycoerythrin (PE)-conjugated mouse monoclonal anti-human EphA2: Supplied as 10 μg of antibody in 1 mL saline containing up to 0.5% BSA and 0.1% sodium azide.

Clone #: 371805

Isotype: mouse IgG_{2A}

Reagents Not Provided

- Flow Cytometry Staining Buffer (Catalog # FC001) or other BSA-supplemented saline buffer.

Storage

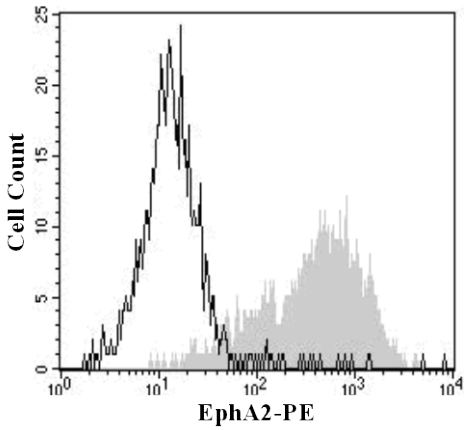
Reagents are stable for **twelve months** from the date of receipt when stored in the dark at 2-8 °C.

Intended Use

Designed to quantitatively determine the percentage of cells bearing EphA2 within a population and qualitatively determine the density of EphA2 on cell surfaces by flow cytometry.

Product Description

This antibody was produced from a hybridoma resulting from the fusion of a mouse myeloma with B cells obtained from a mouse immunized with purified NS0-derived recombinant human Eph Receptor A2 (rhEphA2; aa 25-534; Accession # NP_004422) extracellular domain. The IgG fraction of the tissue culture supernatant was purified by Protein G affinity chromatography. The purified antibody was then conjugated to PE fluorochrome. Cell surface expression of EphA2 is determined by flow cytometry using 488 nm wavelength excitation and monitoring emitted fluorescence with a detector optimized to collect peak emissions at 565-605 nm.



A431 cells were stained with PE-conjugated anti-human EphA2 (Catalog # FAB3035P; filled histogram) or isotype control (Catalog # IC003P; open histogram).

Background Information

EphA2 (also known as Eck) is a 130 kDa type I transmembrane glycoprotein that belongs to the ephrin receptor subfamily of the protein TKR family. Human EphA2 is 952 amino acids (aa) in length and contains a 510 aa extracellular domain (ECD) and a 418 aa cytoplasmic region. The ECD contains one Cys-rich region (aa 188-325) and two fibronectin type III domains (aa 329-526). EphA2 is expressed on epithelium (keratinocytes, renal collecting duct cells, multiple carcinomas) and dendritic/Langerhans cells. It binds to numerous GPI-linked A-class ephrins and likely regulates cell trafficking and localization. The ECD of human EphA2 shares 90%, 93%, and 92% aa sequence identity with the ECD in mouse, canine, and bovine, respectively.

Flow Cytometry Validation

This antibody has been tested for flow cytometry using A431 cells.

1. Cells may be Fc-blocked with 1 μg of human IgG/10^5 cells for 15 minutes at room temperature. Do not wash excess blocking IgG from this reaction.
2. After blocking, 10 μL of conjugated antibody was added to up to 1 x 10^6 cells and incubated for 30 minutes at room temperature.
3. Unbound antibody was removed by washing the cells twice in Flow Cytometry Staining Buffer (Catalog # FC001). Note that whole blood requires a RBC lysis step at this point using Flow Cytometry Human Lyse Buffer (Catalog # FC002).
4. The cells were resuspended in Flow Cytometry Staining Buffer for final flow cytometric analysis. As a control for this analysis, cells in a separate tube should be treated with PE-labeled mouse IgG_{2A} antibody. This procedure may need to be modified, depending upon the cell type and final utilization. Individual users may need to titrate to determine the optimal reagent amount for their specific use.

Warning: Contains sodium azide as a preservative - sodium azide may react with lead and copper plumbing to form explosive metal azides. Flush with large volumes of water during disposal.

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